EXHIBIT A

Aliment. Pharmacol Ther. (1992) **6**, 701–706.

Absorption of polar drugs following caecal instillation in healthy volunteers

S. A. RILEY*, M. KIM†, F. SUTCLIFFE†, M. ROWLAND† & L. A. TURNBERG*

** Epithelial Research Centre, Department of Medicine, Hope Hospital, Manchester and † Department of Pharmacy, Coupland Building III, University of Manchester, UK*

Accepted for publication 16 July 1992

SUMMARY

We have investigated colonic drug absorption in man by the caecal instillation of a multi-component solution of atenolol, cimetidine, frusemide, hydrochlorothiazide and salicylic acid. We found that salicylic acid absorption from this solution was delayed but complete whereas the absorption of atenolol, cimetidine, frusemide and hydrochlorothiazide was four- to five-fold lower than expected from oral bioavailability studies.

INTRODUCTION

There has been growing interest in colonic drug delivery in recent years. Transit through the colon is slow in comparison with other regions of the gastrointestinal tract,[1] and thus slow-release formulations may extend the duration of action of a drug, providing that the drug can permeate the colonic mucosal barrier. However, there has been no systematic study of colonic drug permeation in man and although lipophilic drugs are well absorbed from the colon others are incompletely absorbed.[2]

We have recently studied the absorption of a range of polar drugs in man using

Correspondence to: Dr S. A. Riley, Department of Internal Medicine, Northern General Hospital, Herries Road, Sheffield, S5 7AU, UK.

EXHIBIT A

an intestinal perfusion technique and have found greater absorption from the jejunum than from the ileum. We have therefore studied the absorption of these drugs following caecal instillation in healthy volunteers.

METHODS

Five subjects were studied, two male and three female, with ages ranging from 19 to 33 years. All were in good health and had no gastrointestinal symptoms. No subject had a history of gastrointestinal surgery or known allergy to the study drugs and all were asked to abstain from medication and alcohol in the week prior to study. The protocol was approved by Hope Hospital Ethics Committee and subjects gave written informed consent prior to study.

All five subjects were studied following caecal drug infusion and one subject underwent repeat study following upper jejunal drug infusion. After a 6-h fast subjects swallowed a double-lumen radio-opaque polyvinyl tube incorporating a terminal tungsten weight inside a latex balloon. One lumen supplied the balloon and the other provided an infusion port 1 cm proximal to the balloon. The weight was used to carry the tip of the tube through the pylorus and the balloon was then inflated to encourage forward propulsion of the tube. Intermittent radiographic screening was used to assess the arrival of the infusion port in the caecum or just distal to the duodeno–jejunal flexure. The balloon was then deflated to minimize distal travel of the tube during the infusion period.

The drugs were infused as a multi-component mixture of 20 mg atenolol, cimetidine, 200 mg cimetidine, 20 mg frusemide, 25 mg hydrochlorothiazide and 500 mg salicylic acid, in a 200 ml isotonic electrolyte solution (Na^+ 120 meq/L, Cl^- 120 meq/L, K^+ 30 meq/L, HCO^{3-} 30 meq/L). The solution was infused, by syringe pump, over 60 min and the tube was withdrawn 2 h later.

Blood samples were taken before the start of the infusion and at 10, 20, 30, 45, 60 and 90 min and at 2, 3, 4, 5, 6, 9, 12 and 24 h thereafter. All urine passed in the 24 h following drug infusion was collected. The time from onset of infusion to defaecation was noted.

Plasma and urinary atenolol, cimetidine, frusemide and hydrochlorothiazide concentrations were measured by high-performance liquid chromatography, plasma salicylic acid by a fluorometric method and urinary salicylic acid concentrations by a colorimetric assay as detailed previously.[3]

RESULTS

The procedure was well tolerated in all subjects and the minimum time from drug infusion to defaecation was 8 h (mean 12.1 h, range 8–23).

In the 24 h following caecal infusion the percentage of administered drug excreted in the urine was: atenolol 13.1: (7.0–19.8%), frusemide 7.4% (4.3–11.6%), hydrochlorothiazide 7.1% (5.2–9.2%), cimetidine 9.7% (4.9–17.5%) and salicylic

EXHIBIT A

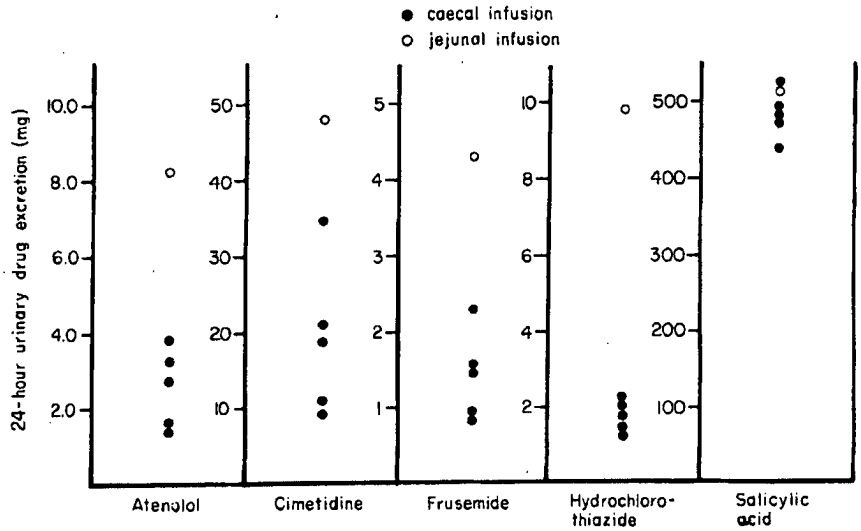

Figure 1. Twenty-four-hour urinary drug excretion following caecal and jejunal infusion of a multi-component solution of 25 mg atenolol, 200 mg cimetidine, 20 mg frusemide, 25 mg hydrochlorothiazide and 500 mg salicylic acid.

Table 1. Plasma kinetics following caecal and jejunal infusion

Drug*	Caecal infusion, $n = 5$ median (range)	Jejunal infusion, $n = 1$
Atenolol		
C_{max} (ng/ml)	21 (10–34)	179
t_{max} (min)	96 (60–240)	120
(AUC (ng. h/L)	0.19 (0.11–0.32)	1.4
Frusemide		
C_{max} (ng/ml)	31 (21–36)	840
t_{max} (min)	290 (240–360)	60
AUC (ng. h/L)	0.22 (0.17–0.25)	0.87
Hydrochlorothiazide		
C_{max} (ng/ml)	17 (7–26)	361
t_{max} (min)	96 (60–120)	120
AUC (ng. h/L)	0.09 (0.03–0.16)	1.1
Cimetidine		
C_{max} (μg/ml)	0.21 (0.12–0.47)	2.8
t_{max} (min)	318 (30–720)	120
AUC (ng. h/L)	0.92 (0.47–1.26)	4.6
Salicylic acid		
C_{max} (μg/ml)	34 (29–39)	52
t_{max} (min)	192 (120–240)	45
AUC (ng. h/L)	323 (234–411)	444

C_{max} – maximum plasma concentration; t_{max} – time to maximum plasma concentration; AUC – area under the plasma drug concentration–time curve (linear trapezoidal rule).

EXHIBIT A

Table 2. Ranking of subjects by urinary drug excretion following caecal infusion

Subject	Atenolol	Frusemide	Hydrochloro-thiazide	Cimetidine	Salicylic acid
A	1	1	1	1	4
B	2	2	2	5	1
C	3	3	3	2	2
D	4	4	5	3	5
E	5	5	4	4	3

acid 97.2% (88.7–103%). Following jejunal infusion the 24-h urinary drug excretion was atenolol 41.2%, frusemide 21.6%, hydrochlorothiazide 39.3%, cimetidine 23.7% and salicylic acid 103% (Figure 1). The corresponding plasma C_{max}, t_{max} and total AUC values following caecal and jejunal infusion are shown in Table 1.

The ranking of individual subjects by 24-h urinary drug excretion following caecal instillation (Table 2) revealed a significant relationship between the extent of colonic absorption of atenolol, frusemide and hydrochlorothiazide and cimetidine (Friedman's Analysis of Variance, $\chi^2 = 11.8$, $P < 0.02$).

DISCUSSION

We have investigated colonic drug absorption in man by the caecal instillation of a multi-component solution containing five drugs. Atenolol, cimetidine, frusemide and hydrochlorothiazide are polar drugs and salicylic acid, incorporated as a test of experimental compliance, is a small lipophilic compound. The drugs are all in common clinical use and are frequently prescribed in combination. There is no evidence of drug interactions within the group, and all, with the exception of salicylic acid, are excreted largely unchanged in the urine so that cumulative urine excretion may be used as an index of absorption. Although salicylic acid is extensively metabolized total urinary salicylates are a quantitative measure of the amount of salicylic acid absorbed. The reported bioavailabilities of these drugs are: atenolol 56 ± 30%, cimetidine $62 + 6$%, frusemide $61 + 17$%, hydrochlorothiazide $71\% + 15\%$ and salicylic acid 100%.[4]

In the present study the absorption of atenolol, cimetidine, frusemide and hydrochlorothiazide following caecal instillation was four- to five-fold lower than that reported following oral administration. The extent of colonic absorption of co-administered salicylic acid, on the other hand, was comparable to that found following oral intake. Moreover, following the single jejunal infusion experiment the extent of absorption of atenolol, cimetidine, frusemide and hydrochlorothiazide was intermediate between published oral data and colonic absorption but salicylic acid absorption was again near complete.

The rates of absorption of atenolol, cimetidine, frusemide and hydro-

EXHIBIT A

chlorothiazide, as judged by C_{max} and t_{max} values, were extremely variable following caecal infusion suggesting erratic colonic absorption. The rate of salicylic acid absorption, however, was more consistent but t_{max} values were considerably delayed, compared with those seen following oral ingestion,[5] even though the rate of caecal infusion was similar to the rate of gastric emptying seen after the oral ingestion of a comparable test solution.[3] This delay may relate to differences in colonic and small intestinal permeability and mucosal surface area, or, because of the larger diameter of the colon, to reduced drug exposure to the available colonic mucosa.

It was interesting to note that, following caecal drug infusion, subjects who absorbed the most atenolol also absorbed the most cimetidine, frusemide and hydrochlorothiazide. The reason for this ranking is not clear but it may reflect inter-subject differences in colonic mucosal exposure to the drugs or in permeability pathways.

Colonic drug absorption in man has been studied by intubation and colonoscopic techniques and by remote-control delivery systems.[6–8] Many of the drugs studies appear well absorbed[2] but drugs such as ciprofloxacin, frusemide, piretanide and nitrofurantoin are poorly absorbed.[9–11]

The relationship between the physical characteristics of a drug and its colonic absorption is not yet clear but studies in the rat suggest that lipophilic drugs are well absorbed along the length of the gastrointestinal tract whereas hydrophilic polar drugs are absorbed much less from the colon than from the small intestine.[12] Similarly, studies in man, using PEG400 as a hydrophilic paracellular permeability probe, have shown greater absorption of this marker from the jejunum than from the ileum and far less absorption from the colon.[13,14] In recent human perfusion studies we have found greater absorption of atenolol, cimetidine, frusemide and hydrochlorothiazide from the jejunum than from the ileum.[3] Poor colonic absorption of polar drugs seen in the present study adds further weight to the concept that regional differences in gastrointestinal drug absorption may reflect variations in paracellular permeability. This has important implications for the development of slow-release formulations which may deliver considerable amounts of drug into the colon.

REFERENCES

1 Hardy J G, Wilson C G, Wood E. Drug delivery to the proximal colon. J Pharm Pharmacol 1985; 37: 874–7.

2 Fara J W. Colonic drug absorption and metabolism. In : Prescott L F, Nimmo W S eds. Novel drug delivery and its therapeutic application. Chichester: John Wiley & Sons, 1989.

3 Riley S A, Kaeser-Laird B, Sutcliffe F, Rowland M, Turnberg L A. Influence of gastrointestinal transit on the permeation of polar drugs in healthy volunteers. Br J Clin Pharmacol 1992; (in press).

4 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Goodman, Gilman A, Rall T W, Nies A S, Taylor P eds Oxford: Pergamon Press, 8th edn, 1990.

5 Rowland M, Riegelman S, Harris P A, Sholkoff S D. Absorption kinetics of aspirin in man following oral administration of an

EXHIBIT A

aqueous solution. Pharm Sci 1972; 61: 379–85.
6 Godbillon J, Evard D, Vidon N, *et al.* Investigation of drug absorption from the gastrointestinal tract of man. III. Metoprolol in the colon. Br J Clin Pharmac 1985; 19: 113S–18S.
7 Gleiter C H, Antonin K H, Bieck P, *et al.* Colonoscopy in the investigation of drug absorption in healthy volunteers. Gastrointestinal Endoscopy 1985; 31: 71–3.
8 Staib A H, Loew D, Harder S, Graul E H, Pfab R. Measurement of theophylline absorption from different regions of the gastro-intestinal tract using a remote-controlled drug delivery device. Eur J Clin Pharmacol 1986; 30: 691–7.
9 Brockmeier D, Grigoleit H G, Leonhardt H. The absorption of piretanide from gastrointestinal tract is site-dependent. Eur J Clin Pharmacol 1986; 30: 79–82.
10 Harder S, Fuhr U, Beerman D, Staib A H. Ciprofloxacin absorption in different regions of the human gastrointestinal tract. Investigations with the HF capsule. Br J Clin Pharmacol 1990; 30: 35–9.
11 Graul E H, Loew D, Schuster O. Voraussetzung für die Entwicklung einer sinnvollen Retard- und Direktika-Kombination. Therapiewoche 1985; 35: 4277–91.
12 Tukker J J, Poelma F G J. Site-dependent absorption in the rat intestine. Third International Conference on Drug Absorption, Edinburgh, 1988.
13 Chadwick V S, Phillips S F, Hofmann A F. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). Gastroenterology 1977; 73: 247–51.
14 Ma T Y, Hollander D, Krugliak P, Katz K. PEG 400, a hydrophilic molecular probe for measuring intestinal permeability. Gastroenterology 1990; 98: 39–46.